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Impact of Phytase Supplementation on Nutrient Digestibility for *Labeo rohita* Fingerlings Fed on Sunflower Meal Based Diets

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Abstract

Sunflower meal based diets, having crude protein (30.70%) and caloric value (4.28 Kcal/g) supplemented with seven graded levels, were fed to *Labeo rohita* fingerlings and compared to a reference diet based on fish meal (30.21% crude protein, 4.26 Kcal/g) in relation to the nutrients digestibility. Chromic oxide (1%) was included as non-digestible marker in the feeds. The sunflower meal based diets became at par with the performance of reference diet at 500 FTU kg^{-1} level. Digestibility coefficients for sunflower meal based diet increased 23.18%, 13.42% and 19.30% for crude protein, crude fat and apparent gross energy as compared to the reference diet, respectively at 750 FTU kg^{-1} level. This study suggested that phytase supplementation to sunflower meal based diets at the 750 FTU kg^{-1} level is enough to release sufficient chelated nutrients for optimal growth performance of *Labeo rohita* fingerlings.

Key words: *Labeo rohita*, Phytase, Nutrient digestibility, Sunflower meal

Introduction

The aquaculture feed industry depends on the use of fishmeal as a source of fundamental nutrients such as amino acids, fatty acids, vitamins, major minerals and growth factors (Zhou et al., 2004). However, rising demand, high price and unstable supply of the fish meal made it compulsory to search for alternative protein sources for aquaculture (Pham et al., 2008). Plant by-products are a promising source of protein and energy (Hardy, 2000; Gatlin et al., 2007) for the formulation of economical and environment friendly aqua-feeds (Cheng and Hardy, 2002). One of the problems related with the use of low cost plant proteins in aqua-feed is the presence of anti-nutritional factors such as phytate or phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexaphosphate) which have deleterious effects on the morphology and physiology of the digestive tract and are known to effect overall fish growth performance (Baruah et al., 2004). It is thought that about 80% of the total phosphorus (P) content in plants are usually found in the form of phytate which is practically not available for agastric or mono-gastric fishes (NRC, 1993). Apart from minerals, phytate also complexes with proteins to decrease the bioavailability of these nutrients in fish (Helland et al., 2006). Phytase supplementation in plant based diets has been used to liberate free phosphorus from phytate complexes (Lim and Lee, 2009). Phytase, an enzyme chemically known as myo-inositol hexaphosphate phosphohydrolase, is produced either by microorganisms or may be present in some plant ingredients. It hydrolyzes the indigestible phytate present in plant proteins. Mono-gastric fishes cannot produce this enzyme and therefore cannot hydrolyze phytate. Supplementation of phytase in fish feeds has been generally reported to improve the bio-availability and utilization of plant phosphorus by fish (Vielma et al., 1998; Baruah et al., 2007; Cao et al., 2007).

Sunflower meal may have up to 40% protein depending on the oil extraction and dehulling techniques (Mushtaq et al., 2006). It has been also recognized that the disruption of cell wall matrix of sunflower meal by supplementation of exogenous microbial enzymes can cause endogenous proteolytic enzymes to digest the chelated proteins (Choct and Kocher, 2000). Study was designed to know the suitability of sunflower meal as a major protein source in practical and economical diets for commercially important species of carps like *Labeo rohita*. Such new formulations will not only reduce fish production costs but will also help to solve aquatic pollution problems by reducing the liberation of phytate phosphorus rich excreta.

Materials and Methods

The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

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Fish and experimental conditions

Labeo rohita fingerlings (Avg. wt. 7.05g) were obtained from Public Hatchery, and allowed to acclimatize in the laboratory for two weeks in V-shaped tanks (UA system) having 70L water capacity, specially designed for the collection of fecal material from V- shaped water tanks. During this period the fingerlings were fed once daily to apparent satiation on the basal diet (Allan and Rowland, 1992). Water quality variables, particularly water temperature, pH and dissolved oxygen were monitored with a Jenway 3510 pH meter and Jenway 970 D.O. Meter. Twenty-four hour aeration was provided to all the tanks through a capillary system. Before starting the experiment, *Labeo rohita* fingerlings were treated with (5g/L) NaCl to remove ectoparasites and to prevent fungal infection (Rowland and Ingram 1991).

Feed ingredients and experimental diets

The feed ingredients were purchased from market and analyzed for chemical composition following standard procedures (AOAC, 1995) prior to the formulation of the experimental diets (Table 1). The reference diet was prepared to supply adequate levels of required nutrients for normal fish growth. Chromic oxide was used as an inert marker at 1% inclusion level in the reference diet. The test diet was composed of 70 % reference diet and 30 % sunflower meal (Table2). The feed ingredients were finely ground to pass through 0.5 mm sieve size. All ingredients were mixed in an electric mixer for 10 minutes and fish oil was gradually added along with 10-15 percent water to provide moisture. Floating pellets (3 mm) were prepared using an Experimental Lab Extruder (model SYSLG30-IV). The phytase (Phyzyme® XP 10000 FTU/g; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) stock solution was prepared by dissolving 2g of microbial phytase (powder form) in 1000 ml of distilled water (Robinson et al., 2002). Seven sub-test diets were then prepared by spraying graded levels (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg^{-1} levels) of phytase into sunflower meal based diet. One unit of phytase activity (FTU) is defined as the enzyme activity that liberates 1 µmol of inorganic orthophosphate min^{-1} at pH 5.5 at a temperature of 37^{o}C and a substrate concentration (sodium phosphate) of 5.1 µmol/L (Engelen et al., 1994).

Feeding Protocol and Sample Collection

Labeo rohita fingerlings were fed twice daily (morning and afternoon) to apparent satiation. Initially, the fingerlings were fed at the rate of 2 % of live wet weight on their prescribed diet and subsequently adjusted on daily feed intake. For each test diet three replicates were assigned and in each replicate fifteen fish were stocked. After the feeding session of three hours, the uneaten diet was drained from each tank by opening the valves of the tanks. The tanks were washed completely to remove the particles of diets and refilled with water. After this, feces were collected from the fecal collection tube of each tank by opening the valve I and valve II (situated below the fecal collection tube) subsequently. Care was taken to avoid breaking the thin fecal strings in order to minimize nutrient leaching. Fecal material of each replicated treatment was dried in oven 60°C for 24 hours, ground and stored for chemical analysis. The experiment continued for ten weeks and 4-5 g of fecal material was collected from each replicate tank.

Chemical Analysis of Feed and Feces

The samples of feed ingredients, test diets and feces were homogenized using a motor and pestle and analyzed by standard methods (AOAC, 1995): moisture was determined by oven-drying at 105^{o}C for 12 h; crude protein (N x 6.25) by micro kjeldahl apparatus; crude fat, by petroleum ether extraction (Bligh and Dyer, 1995) using a Soxtec HT2 1045 system; crude fiber, as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH, ash by ignition at 650^{o}C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight. Total carbohydrates (N-free extract) were calculated by difference as follows:

Table 1 Chemical composition (%) of feed ingredients

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Total Carbohydrate (%)	Gross Energy (kcal/g)
Fish meal	91.63	48.15	7.16	0.52	26.23	17.94	3.69
Wheat flour	92.45	10.10	2.35	1.65	2.08	83.82	2.96
Corn gluten 60%	92.59	59.12	4.96	1.19	1.58	33.15	4.23
Rice polish	94.09	12.35	12.31	2.71	7.90	64.73	4.33
Sunflower meal	94.13	42.91	3.27	1.74	10.90	41.18	3.63

Table 2 Ingredients composition (%) of reference and test diets

Ingredients	Reference diet	Test diets
Fish meal	20.0	14.0
Wheat flour	24.0	16.8
Corn gluten 60%	20.0	14.0
Rice polish	25.0	17.5
Fish oil	7.0	4.9
Vitamin Premix*	1.0	0.7
Minerals	1.0	0.7
Ascorbic acid	1.0	0.7
Chromic oxide	1.0	0.7
Sunflower meal	-	30.0

Total carbohydrate (%) = 100- (CP% + EE% + Ash% + CF%). Gross energy was determined by oxygen bomb calorimeter. Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent (Divakaran et al., 2002) using a UV-VIS 2001 Spectrophotometer at 370nm absorbance.

Calculation of digestibility coefficients

Apparent nutrient digestibility coefficients (ADC) of test diets were calculated by the formula reported in NRC (1993):

$$ADC\ (\%) = 100 - 100 \times \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} \times \text{Percent nutrient in diet}}$$

Statistical Analysis

Data of nutrients digestibility of experimental diets was subjected to one-way analysis of variance, ANOVA (Steel et al., 1996) The differences among means were compared by Tukey's honesty significant difference test and considered significant at $P<0.05$ (Snedecor and Cochran, 1991). The CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results

The apparent nutrient digestibility (%) for reference and sunflower meal based test diets are shown in Table 3. It was obvious from the results that, in comparison with reference diet, sunflower meal based test diets supplemented with phytase enzyme released less nutrients through feces at 750 and 1000 FTU kg^{-1} levels. Maximum crude protein, crude fat and ADC (%) coefficients were observed at 750 followed by 1000 FTU kg^{-1} levels. These values were not only significantly different from each other but significantly different ($P<0.05$) from the remaining diets. It was interesting to observe that below 750 and above 1000 FTU kg^{-1} levels, phytase supplementation could not perform equivalent to the reference diet in terms of crude protein and crude fat digestibility. However, the results clearly indicate that ADC(%) increased significantly ($P<0.05$) at 500 FTU kg^{-1} level as compared to 0 FTU kg^{-1} level. However, test diet with 750 FTU kg^{-1} of phytase was found to be optimal for *Labeo rohita* (Table 3).

Discussion

Phytate binds with trypsin thus reducing the bioavailability of protein components and overall performance in certain fish feed formulations (Tacon, 1997). In the present study, *Labeo rohita* fingerlings fed on a test diet supplemented with 0 and 250 FTU kg^{-1} levels performed non-significantly different to each other as well as to the reference diet hinting that these levels of phytase supplementation were not enough to liberate the bound form of protein-phytate complexes. However, the poor performance at higher phytase levels is difficult to explain. However Baruah et al. (2007) also observed similar trend. The highest ADC (%) of *Labeo rohita* fingerlings fed on sunflower meal based diet was observed at 750 FTU kg^{-1} level, while the next higher digestibility observed at 1000 FTU kg^{-1} level. An increasing trend of crude protein digestibility was reported by Baruah et al. (2007). They found that maximum digestibility of crude protein for *Labeo rohita* juveniles was reported at 750 FTU kg^{-1} level, then there was a decrease in crude protein digestibility on soybean meal based diet at 1000 FTU kg^{-1} level. In a comparative study with two different types of phytase enzyme, Liebert and

Table 3 Apparent crude digestibility of nutrients (%) of reference and sunflower meal based diets

Diets	Phytase levels (FTU kg^{-1})	Crude Protein (%)	Crude Fat (%)	Apparent gross energy digestibility (%)
Reference diet	--	58.88 ± 1.02^{cd}	69.02 ± 0.64^{c}	61.05 ± 0.89^{cd}
Test diet I	0	55.12 ± 0.29^{e}	65.86 ± 0.48^{de}	52.87 ± 0.55^{f}
Test diet II	250	57.62 ± 2.12^{cde}	67.93 ± 0.29^{cd}	59.19 ± 1.13^{de}
Test diet III	500	59.68 ± 0.99^{c}	73.99 ± 1.37^{b}	62.97 ± 1.20^{c}
Test diet IV	750	72.53 ± 0.58^{a}	78.28 ± 0.97^{a}	72.83 ± 1.07^{a}
Test diet V	1000	66.42 ± 1.62^{b}	74.36 ± 1.58^{b}	69.11 ± 0.84^{b}
Test diet VI	1250	58.82 ± 1.55^{cd}	66.32 ± 0.63^{cde}	58.82 ± 1.09^{de}
Test diet VII	1500	55.42 ± 1.01^{de}	63.25 ± 1.80^{e}	56.78 ± 1.85^{e}

Portz, (2007) also reported that 750 FTU kg^{-1} level of phytase-A (SP 1002) supplementation was adequate for improving nutrient digestibility that resulted in better fish growth while the phytase-B (Ronozyme® P) with 1000 FTU kg^{-1} level supplementation was found sufficient for maximum phytate degradation resulting in increased nutrient digestibility and growth performance. The higher ADC of crude protein observed in the present experiment agrees with the acceptability of the alternative plant protein based test diets supplemented with phytase enzyme as reported by Nwanna et al, (2008). In another study, Furuya et al. (2001) determined optimal phytase supplementation levels ranging from 500 to 1500 FTU kg^{-1} in Nile tilapia (*Oreochromis niloticus*). These studies suggest that nutrient digestibility may be associated with variations in the source of phytases or other factors, such as protein quality of feed ingredients, pH of fish stomach and feed formulation procedures (Wang et al., 2009).

The highest value of crude fat digestibility was also observed at 750 FTU kg^{-1} level which was 23.18% higher than the reference diet. Portz and Liebert (2004) reported significant improvement in crude fat digestibility for diets supplemented with phytase at 1000 and 2000 FTU kg^{-1} levels. Ashraf and Goda, (2007) also indicated that in experimental diets, phytase supplementation improved the apparent digestibility coefficients of lipid at 1000 FTU kg^{-1} level. However, in another study, Dalsgaard et al. (2009) found no significant effect on fat digestibility in rainbow trout (*Oncorhynchus mykiss*) fed plant based diets supplemented with phytase. On the other hand, Wang et al. (2009) reported reduced lipid digestibility by phytase supplementation. They had the opinion that phytase supplementation may inhibit lipase activity by reducing lipase hydrolysis efficiency. Highest digestibility of gross energy was also observed at the 750 FTU kg^{-1} level. The findings in the present study are in agreement with Forster et al., (1999) who reported an increased apparent gross energy digestibility from phytase supplemented canola protein concentrate. The use of phytase enzymes in the diet of tambaqui (*Colossoma macropomum*) enhanced nutrient digestibility and gross energy (Moreira et al., 2007). Ashraf and Goda (2007) also indicated that in experimental diets phytase supplementation at 1000 FTU kg^{-1} level improved the apparent digestibility coefficient of gross energy. Improved gross energy digestibility due to phytase supplementation resulted in better fish growth performance (Debnath et al., 2005). Cheng and Hardy (2002) reported that phytase supplementation of diets having canola protein concentrate improved apparent gross energy digestibility for rainbow trout, while Lanari et al. (1998) did not observed a positive response for rainbow trout fed soybean meal based test diets. This suggested that the effect of phytase supplementation on the gross energy utilization may be dependent on feed and phytase sources, feed processing techniques and fish species.

References

Allan GL and SJ Rowland, 1992. Development of an experimental diet for silver perch (*Bidyanus bidyanus*). Austasia Aquaculture, 6: 39-40.

Ashraf M and AS Goda, 2007. Effect of dietary soybean meal and phytase levels on growth, feed utilization and phosphorus discharge for Nile tilapia (*Oreochromis niloticus* L.). Journal of Fisheries Aquatic Sciences, 2: 248-263.

Association of Official Analytical Chemists (AOAC), 1995. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists, Washington, D.C. USA., p. 1094.

Baruah K, KAK Pal, PS Narottam and D Debnath, 2007. Microbial Phytase supplementation in rohu, *Labeo rohita*, diets enhances growth performance and nutrient digestibility. Journal of World Aquaculture Society, 38: 129-137.

Baruah, K, NP Sahu, AK Pal and D Debnath, 2004. Dietary Phytase: An ideal approach for cost effective and low-polluting aquafeed. NAGA, World Fish Center Quarterly, 27: 15-19.

Bligh EG and WJ Dyer, 1995. A rapid method of total lipid extraction and purification. Can. Journal of Biochemistry and Physiology, 37: 911-917.

Cao L, W Wang, C Yang, Y Yang, J Diana, A Yakupitiyage, Z Luo and D Li, 2007. Application of microbial phytase in fish feed. Enzyme Microbial Technology, 14: 342-362.

Cheng ZJ and RW Hardy, 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured in vivo using rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, 8: 271-277.

Choct M and A Kocher, 2000. Use of enzymes in non cereal grain feedstuffs. Proc. XXI World's Poult. Congr. WPSA, Montreal, Canada.

Dalsgaard J, KS Ekmann, PB Pedersen and V Verlhac, 2009. Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-

depleted juvenile rainbow trout (*Oncorhynchus mykiss*) and on the magnitude and composition of phosphorus waste output. Aquaculture, 286: 105-112.

Debnath D, AK Pal, PS Narottam, KK Jain, S Yengkokpam and SC Mukherjee, 2005. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquaculture Research, 36: 180-187.

Divakaran S, GO Leonard and PF Ian, 2002. Note on the methods for determination of chromic oxide in shrimp feeds. Journal of Agriculture and Food Chemistry, 50: 464-467.

Engelen AJ, FC Van-der Heeft, PHG Randsdrop and ELC Smith, 1994. Simple and rapid determination of phytase activity. Journal of the Association of Official Analytical Chemists, 77: 760-764.

Forster I, DA Higgs, BS Dosanjh and M Rowshandeli, 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 ◦C fresh water. Aquaculture, 179: 109-125.

Furuya WM, GS Goncalves, V.R.B. Furuya and C. Hayashi. 2001. Phytase as feeding for Nile tilapia, *Oreochromis niloticus*. Performance and digestibility. Revista Brasileira de Zootecnia, 30: 924-929.

Hardy RW, 2000. New developments in aquatic feed ingredients and potential of enzyme supplements. In: Advances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola, Merida, Yucatan, Mexico, 19-22 November (Ed. By L.E. Cruz-Suarez, D. Ricque-Marie, M.Tapia-Salazar, M.A. Olvera-Novoa & R. Civera-cerece-do). p: 216-226.

Helland S, V Denstadli, PE Witten, K Hjelde, T Strobakken, A Skrede, T Asgard and G Baeverfjord, 2006. Hyper dense vertebrae and mineral content in Atlantic salmon (*Salmo salar* L) fed diets with graded levels of phytic acid. Aquaculture, 261: 603-614.

Lanari D, ED Agaro and C Turri, 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 161: 345-356.

Liebert F and L Portz, 2007. Different sources of microbial phytase in plant based low phosphorus diets for Nile tilapia *Oreochromis niloticus* may provide different effects on phytate degradation. Aquaculture, 267: 292-299.

Lim SJ and KJ Lee, 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture, 290: 283-289.

Moreira da-Silva, JA, M Pereira-Filho, BAS Cavero and MI de-Oliveira-Pereira, 2007. Apparent digestibility of nutrients and crude energy in diets with addition of exogenous digestive enzymes in tambaqui juveniles (*Colossoma macropomum* Cuvier,1818). Acta Amazon, 37: 157-164.

Mushtaq T, M Sarwar, G Ahmad, MU Nisa and A Jamil, 2006. The influence of exogenous multi-enzyme preparation and graded levels of digestibility sine on the performance of young broiler chicks two weeks post hatching in sunflower meal based diets. Poultry Science, 85: 2180-2185.

National Research Council (NRC), 1993. Nutrient Requirements of Fish. Washington, DC, National Academy Press, p. 114.

Nwanna LC, CA Oishi and MP Filho, 2008. Use of phytase to improve the digestibility of alternative feed ingredients by Amazon tambaqui, *Colossoma macropomum*. Science Asia, 34: 353-360.

Pham MA, KJ Lee, TM Dang, SJ Lim, GY Ko, J Eo and DH Oh, 2008. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*). Asian-Australas. Journal of Animal Sciences, 21: 1367-1375.

Portz L and F Liebert, 2004. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant based diets with graded levels of microbial phytase. Journal of Animal Physiology and Animal Nutrition, 88: 311-320.

Rowland SJ and BA Ingram, 1991. Diseases of Australian native fishes. In: Fisheries Bulletin 4 NSW Fisheries, Sydney, NSW, Australia.

Snedecor GW and WG Cochran, 1991. Statistical Methods. 8th Ed. Iowa State Univ. Press, Ames. USA, p. 503.

Steel RGD, JH Torrie and DA Dickey, 1996. Principles and Procedures of Statistics, 3rd Ed. McGraw Hill International Book Co. Inc., New York. USA, p. 336-352.

Tacon AGJ, 1997. Fish Meal Replacers: Review of Anti nutrients Within Oilseeds and Pulses-a Limiting Factor for the Aquafeed Green Revolution? In: Feeding tomorrow's fish. Tacon, A. & B. Basurco (Eds.), Proceedings of the workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM, FAO and IEO Mazarron, Spain, 24-26 June 1996, CIHEAM, Apodo, Spain, pp: 153-182.

Vielma J, SP Lall and J Koskela, 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 163: 309-323.

Wang F, YH Yang, ZZ Han, HW Dong, CH Yang and ZY Zou, 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture International, 17: 143-157.

Zhou QC, BP Tan, KS Mai and YJ Liu, 2004. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. Aquaculture, 241: 441-451.